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AUTHORITY

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Protection Branch Report of Test No. 7-60

A TECHNIQUE FOR THE INVESTIGATION OF BACTERIAL CONTAMINATION INSIDE ELECTRONIC COMPONENTS

11 March 1960

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Protection Branch Report of Test [redacted]-7-60

A Technique for The Investigation of Bacterial Contamination Inside Electronic Components.

(11) 11 Mar 60

INTRODUCTION

(12) 13p.

The advent of space rocketry has posed the problem of sterilizing probes, ~~prior to launchings,~~ which might impact an extra terrestrial body. Contamination, and possible infection, of these bodies with earth microorganisms could interfere with or completely invalidate subsequent biological investigations, and unique opportunities to investigate the origin of life on other planets ~~could be lost~~ AN IMPACTED forever. A "hard hit" by a missile on the moon or one of the planets could result in shattering the probe and all its components. For this reason the missile and all its components must be sterile not only externally but internally as well.

For several reasons, the main one being that a variety of sensitive materials would be damaged by other techniques, ethylene oxide gas will be used to sterilise the final assembled payload just prior to launching. This gas has remarkable penetrating capabilities but it can not enter hermetically sealed areas, as can heat or certain ionizing radiations. Many of the newer electronic components are sealed units into which the gas can not penetrate, but in which living microorganisms may have been trapped during manufacture. Thus, we do not know whether surface sterilisation is all that is required for such devices. The lack of information on the subject prompted this investigation. The main purpose of this ~~preliminary~~ study was to develop satisfactory techniques for such investigations, following which routine investigations of all types of components could ~~be made. In~~ the course of this study certain electronic components were screened for possible internal contamination, but only a few items were so tested, and the fact that a few types were found to be sterile should not be interpreted as a statistically valid study on this subject.

DETECTING WHETHER OR NOT MICROORGANISMS MAY HAVE BEEN TRAPPED DURING THE MANUFACTURING PROCESS WITHIN ELECTRONIC COMPONENTS WHICH WOULD PRESENT A CONTAMINATING SOURCE UPON BREAKING APART AT IMPACT. HERMETICALLY SEALED ELECTRONIC COMPONENTS ARE ESPECIALLY CONSIDERED.

~~MATERIALS AND METHODS~~

In order that all work could be performed in an uncontaminated atmosphere, the tests were performed inside an airtight plastic chamber (Figure 1), the interior of which could be kept sterile. The chamber is fitted with glove ports and rubber gloves, a screw type door and small air inlet tubes. In general, the test procedure involved placing in the chamber, the electronic components, broth blanks, forceps, hammers, mortars and pestles, metal hammering block and a can of ethylene oxide-freon mixture. In later tests metal saws and files were also placed in the chamber. The chamber was closed and the ethylene oxide released in order to sterilise the exterior of all the items and the atmosphere in the chamber. Following an 8-hour exposure to ethylene oxide, air sterilised by filtration through a cotton collector was passed through the chamber for 16 hours to remove the oxide. The following bacteriological tests were then performed.

401 661

A pair of each type of electronic component being investigated was placed in the chamber. After the ethylene oxide treatment, one component from each pair was placed, whole, in an individual broth blank. These served as controls, indicating that the exterior surfaces were indeed sterilized by the ethylene oxide treatment. The other component was then broken, ground as well as possible, and the pieces placed in another broth blank. After being well sealed the broth blanks were removed from the chamber and incubated 4-6 days and then examined for cloudiness which might indicate bacterial growth. The bottles were then opened and aliquots of the broth were streaked on tryptose agar to check further for bacterial growth. Following this, each broth blank was seeded with one drop of a 24 hour tryptose broth culture of Staphylococcus aureus and after 24 hours incubation an aliquot of the broth was streaked on tryptose agar. This last step was taken to assure ourselves that the blank was still capable of supporting microbial growth even though no growth had occurred in it when the electronic component was added. Positive growth in this step indicated that the material of which the component was made was not bacteriostatic or bactericidal.

The first and second experiments were performed with electronic tubes, diodes, condensers and resistors obtained from Physical Detection Branch, Physical Defense Division. The third experiment was performed with various types of transistors obtained from Jet Propulsion Laboratories. In this experiment one or two of each type transistor was reserved for electronic tests. These particular components were tested by Pfc John Atherly, Physical Detection Branch, before and after exposure to ethylene oxide to determine if ethylene oxide had any adverse affect on their functionability. The procedure and the results of the functionability test are given in Appendix I. The results of all bacteriological tests are shown in Tables I - IV.

The question arose, after Test 1, whether sufficient ethylene oxide might have penetrated into the tryptose broth under the metal cap of the bottles to prevent the growth of any bacteria introduced into the broth with the addition of an electronic component. Ethylene oxide intially present could easily dissipate from the broth during the 4 to 6 days of incubation and bacteria added thereafter could then multiply. Test 2 was performed using broth blanks (glass bottles with metal screw caps) the same as in test 1. Also included in test 2 were a second set of the same type bottles with electricians tape wound around the caps to insure a tight seal. Both sets of blanks were exposed to ethylene oxide for 8 hours, the chamber then aerated 16 hours and one drop of a 24 hour broth culture of Staphylococcus aureus added to each of six control bottles to determine if the broth was still capable of supporting bacterial growth.

RESULTS

No evidence of microbial contamination, of the electronic components tested, was demonstrated in the bacteriological tests performed. Some of the broth blanks containing the components were suspect of growth because of cloudiness. Subsequent plating on tryptose agar, however, demonstrated the absence of microorganisms. The cloudiness was apparently of chemical rather than biological

origin, caused by materials in the components being tested. The results also showed that the broth was still capable of supporting bacterial growth after 4-6 days incubation with the various electronic components, although in at least one case the growth was not of normal vigor.

The results of one of the untaped control blanks in test 2 showed it was possible for ethylene oxide to penetrate under the bottle cap and into the broth in sufficient amount to inhibit growth of microorganisms present. Thus, the results in test 1 cannot necessarily be relied upon as giving a true picture of the internal contamination of electronic components.

The results of electronic tests indicated that the ethylene oxide treatment had no adverse effect on the functionability of the transistors tested.

DISCUSSION

A procedure to determine the presence of microorganisms inside electronic components has been devised. Inside a completely enclosed system, where all surfaces are sterile, electronic components can be hammered, ground, sawed or filed so that their interiors are exposed. The pieces from the interior can then be placed in sterile broth blanks without ever leaving the enclosed system.

The conditions of test are such, with all manipulations being carried out inside a sealed sterile cabinet, that the possibility of false positives due to a break in sterile technique are virtually excluded. With the change made following test 1, when the bottle caps on the broth blanks were not only screwed but taped shut so that no ethylene oxide could penetrate and render the broth bacteriostatic, it was felt that the possibility of failing to detect microorganisms if indeed present inside the electronic devices, was minimal. One difficulty with the test still remains however. Should one object be internally contaminated, cracking or sawing this open could contaminate the interior of the cabinet and subsequent test components in the run. Thus if 20 objects were being tested in one run and the 10th, 12th and 15th broth blanks showed growth, we would be convinced that all the objects which did not contaminate the broth blanks, were truly sterile, and that the object placed in the 10th blank was truly contaminated. We would have no way of knowing however whether the 12th and 15th objects were themselves contaminated, or whether some organisms from object 10 had been accidentally transferred into these blanks. Thus any contaminated blanks obtained after the one where contamination first shows up, would be suspect.

The restriction set up for probes to Venus, Mars, etc., is that each probe and all its components will be sterile. For this reason, if we find even one of 20 of any particular component contaminated internally, sterilisation of the component by some treatment other than ethylene oxide will be necessary. On the other hand if we find 20 out of 20 of a particular component not contaminated we would be inclined to say no additional treatment is necessary to insure sterility of the interior of this type component.

The possibility of contamination being introduced from a single contaminated object however, places some restriction on how tests in such a cabinet should be conducted. Obviously one should keep accurate records of the order in which each object was broken up and placed in a blank, as well as records of which object went into which blank. Then one should utilize as much as possible the techniques of sterile transfer, even though the work is done in a completely enclosed environment. A sufficient number of sterile forceps, etc., should be placed in the cabinet so that a separate one can be used for each object. The forceps should be touched only on the end which does not touch the object being tested. In other words the hand should be treated as contaminated even though one is working through the barrier formed by sterile gloves. In certain cases, i.e. where an object is to be broken up with a hammer and anvil, it may not be possible to get 20 hammers and 20 anvils in the cabinet, but one can use different areas of the anvil, and separate forceps to pick up the shattered pieces of the object being tested. The value of the entire technique lies in the almost virtual surity that if all objects in one test are sterile on the interior as well as on the exterior, this will be clearly demonstrated, and that if as much as one object is not sterile this will be shown too. The fact that one could over-estimate the per cent contaminated objects in a mixed lot of contaminated and sterile ones is of lesser importance.

None of the components tested were found to be contaminated internally, however, so few items of each type were evaluated that no conclusion can be made at this time regarding the internal sterility of electronic components in general.

Tests of the functionability of transistors before and after treatment with ethylene oxide gas indicate the gas had no adverse effect on the functionability of these components. It is imperative that all types of electronic components, which will be used in missile payloads that are to be sterilized by ethylene oxide, be subjected to similar functionability tests to determine whether the oxide has a detrimental effect on any of the components.

Test 1

Table I

Investigation of Microbial Contamination Inside Various Electronic Components

Component	Condition When Placed In Broth	A Broth After 6 Days @ 37°C	B Growth After 0.1 ml Of A Spread On Tryptose Agar	C 4-24 hr After Adding 1 Drop Of S. aureus Culture	D Growth After 0.1 ml Of C Spread On Surface Of Tryptose Agar
Sylvania Electron Tube 6AL5	Unbroken	Clear	-	Cloudy	+ + +
RCA Electron Tube 6AS6	Broken & Ground	Cloudy	-	Cloudy	+ + +
Sprague Condenser 002MFD, 600 VDC	Unbroken	Clear	-	Cloudy	+ + +
Sangamo Condenser 002MFD, 600 VDC	Broken & Ground	Slightly Cloudy	-	Cloudy	+ + +
Cornell Dubiliar Type 5 Condenser 250 MMF	Unbroken	Clear	-	Cloudy	+ + +
Cornell Dubiliar Type 5 Condenser 250 MMF	Broken & Ground	Clear	-	Cloudy	+ + +
Resistor (Small)	Unbroken	Clear	-	Cloudy	+ + +
Resistor (Small)	Broken	Clear	-	Cloudy	+ + +
Diode Sylvania IN 38A	Unbroken	Clear	-	Cloudy	+ + +
Diode Sylvania IN 38A	Broken	Clear	-	Cloudy	+ + +
Cornell-Dubiliar Condenser (Large)	Broken	Clear	-	Slightly Cloudy	+
Sangamo Condenser Dry Electrolytic	Unbroken	Cloudy	-	Cloudy	+ + +
Glassmike Oil Filled Condenser ASG 20, 001MFD, 3000 WVDC	Broken	Slightly Cloudy	-	Cloudy	+ + +

- indicates no bacterial growth, + indicates light bacterial growth, + + + indicates heavy bacterial growth

Test 2

Table II

Ability of Tryptose Broth to Support Bacterial Growth* After Exposure in Screw Cap Bottles To Ethylene Oxide Gas for Eight Hours

Broth Controls	Type Bottle	Tape Around Bottle Cap	A Broth 24 hour After Inoculation	B Growth After 0.1 ml Of A Spread On Surface Of Tryptose Agar
Not Exposed to ETO	Wide Mouth	No	Cloudy	+ + +
Not Exposed to ETO	Small Mouth	No	Cloudy	+ + +
Exposed to ETO	Wide Mouth	No	Slightly Cloudy	+
Exposed to ETO	Small Mouth	No	Cloudy	+ + +
Exposed to ETO	Small Mouth	No	Cloudy	+ + +
Exposed to ETO	Wide Mouth	Yes	Cloudy	+ + +
Exposed to ETO	Small Mouth	Yes	Cloudy	+ + +
Exposed to ETO	Small Mouth	Yes	Cloudy	+ + +

* 1 drop of 24 hour S. aureus culture added to each broth blank after 8 hours exposure to ethylene oxide and 16 hours aeration of the chamber.

\+ Light bacterial growth

+++ Heavy bacterial growth

Test 2

Table III

Investigation of Microbial Contamination Inside Various Electronic Components

Component*	Type Bottle	Tape Around Bottle Cap	A Broth After 6 Days @ 37°C	B Growth After 0.1 ml Of A Spread on Tryptose Agar	C A-24 hr After Adding 1 Drop Of S. aureus Culture	D Growth After 0.1 ml Of C Spread On Tryptose Agar
Resistor, medium size	Small Mouth	Yes	Clear	-	Cloudy	+++
Resistor, small size	Small Mouth	Yes	Clear	-	Cloudy	+++
Diode, Sylvania IN [illegible]A	Small Mouth	Yes	Clear	-	Cloudy	+++
Electron Tube, RCA 6AS6	Small Mouth	Yes	Cloudy	-	Cloudy	+++
Condenser, Sangamo 002 MFD, 600 VDC	Small Mouth	Yes	Cloudy	-	Cloudy	+++

* All components broken before placing in tryptose broth blanks

\- No bacterial growth

+++ Heavy bacterial growth

Table IV

Investigation of Microbial Contamination Inside Various Type Transistors

Transistor	Condition When Placed in Broth	A Broth After 4 Days @ 37°C	B Growth 24 hrs After 0.1 ml Of A Spread On Tryptose Agar	C A-24 hr After Adding 1 Drop Of S. aureus Culture	D Growth 48 hr After 0.1 ml C Spread On Tryptose Agar
None - Control	-	Cloudy*	+++	Cloudy	+++
None - Control	-	Cloudy*	+++	Cloudy	+++
None - Control	-	Clear	-	Cloudy	+++
Delco 2N278 839	Unbroken	Cloudy	-	Cloudy	+++
Delco 2N278 839	Broken	Cloudy	-	Cloudy	+++
Delco 2N173 838	Unbroken	Cloudy	-	Cloudy	+++
Delco 2N173 838	Broken, Top Only	Cloudy	-	Cloudy	+++
Delco 2N173 838	Broken, Bottom Only	Cloudy	-	Cloudy	+++
T ST 401	Unbroken	Clear	-	Cloudy	+++
T ST 401	Broken	Cloudy	-	Cloudy	+++
T ST 401	Broken	Cloudy	-	Cloudy	+++
Texas 2N332 (black)	Unbroken	Clear	-	Cloudy	+++
Texas 2N332 (black)	Broken	Slightly Cloudy	-	Cloudy	+++
Texas 2N332 (black)	Broken	Slightly Cloudy	-	Cloudy	+++
Ray 2N417 (silver)	Unbroken	Slightly Cloudy	-	Cloudy	+++
Ray 2N417 (silver)	Broken	Cloudy	-	Cloudy	+++
Ray 2N417 (silver)	Broken	Cloudy	-	Cloudy	+++
Ray 2N327 (red)	Unbroken	Clear	-	Cloudy	+++
Ray 2N327 (red)	Broken	Clear	-	Cloudy	+++
Ray 2N327 (red)	Broken	Slightly Cloudy	-	Cloudy	+++

* 1 drop *S. aureus* culture added immediately after exposure to ETO

\- No bacterial growth

+++ Heavy bacterial growth

APPENDIX I

by
Pfc John Atherly

e transistors under test were checked for (1) Beta (the small sig ain), (2) Ico (the collector-to-base leakage current with the emi open) and (3) Io (the collector to emitter leakage current with the e open). These values were measured quantitatively, for any selected voltage (Voc) which is the voltage across the transistor. The transistors were also checked for shorts between the collector-base and collector-emitter. There were no shorts present.

All tests were made on Western Instruments Transistor Tester Model 61. Two tests were run on each transistor before exposure to ethylene oxide gas and one test was run on each after exposure to the gas. The results of these tests are shown in Table V.

Table V

Characteristics of Transistors Before and After Exposure to Ethylene Oxide Gas

Type Transistor	Vcc Volts	Base Current μa	Beta (Current Gain Ma)	Ico μa	Io μa
Ray 2N417 (1)					
Before ETO Exposure	6	100	167	1	-
Before ETO Exposure	6	100	160	2.1	-
After ETO Exposure	6	100	166	2.1	160
Ray 2N417 (2)					
Before ETO Exposure	6	68	184	2.5	-
Before ETO Exposure	6	68	178	2	-
After ETO Exposure	6	68	188	3	-
Texas Ins. 2N332 (1)					
Before ETO Exposure	20	135	22	0	0
Before ETO Exposure	20	135	24	0	0
After Eto Exposure	20	135	22	0.5	0
Texas Ins. 2N332 (2)					
Before ETO Exposure	20	135	19	1	0
Before ETO Expoure	20	135	18	0.5	0.5
After ETO Exposure	20	135	18	1	1
Ray 2N327 (1)					
Before ETO Exposure	24	100	18	0	0
Before ETO Exposure	24	100	17	0	0
After ETO Exposure	24	100	16	0.5	1
Ray 2N327 (2)					
Before ETO Exposure	24	100	13	0	0
Before ETO Exposure	24	100	13	0	0
After ETO Exposure	24	100	12	0	0
ST-401 (1)					
Before ETO Exposure	6	100	2	70	87
Before ETO Exposure	6	100	1	69	86
After ETO Exppsure	6	100	2	69	86
ST-401 (2)					
Before ETO Exppsure	6	100	1.5	2	1
Before ETO Exposure	6	100	0	0.5	0
After ETO Exposure	6	100	0	1	2

Table V (Continued)

Type Transistor	Vcc Volts	Base Current µa	Beta (Current Gain Ma)	Ico µa	Io µa
Delco 2N173					
Before ETO Exposure	7	100	194	Off Scale	
Before ETO Exposure	7	100	196	Off Scale	
After ETO Exposure	7	100	176	Off Scale	
Delco 2N278					
Before ETO Exposure	7	100	157	Off Scale	
Before ETO Exposure	7	100	158	Off Scale	
After ETO Exposure	7	100	144	Off Scale	

(1), (2) Indicate tests on two different transistors

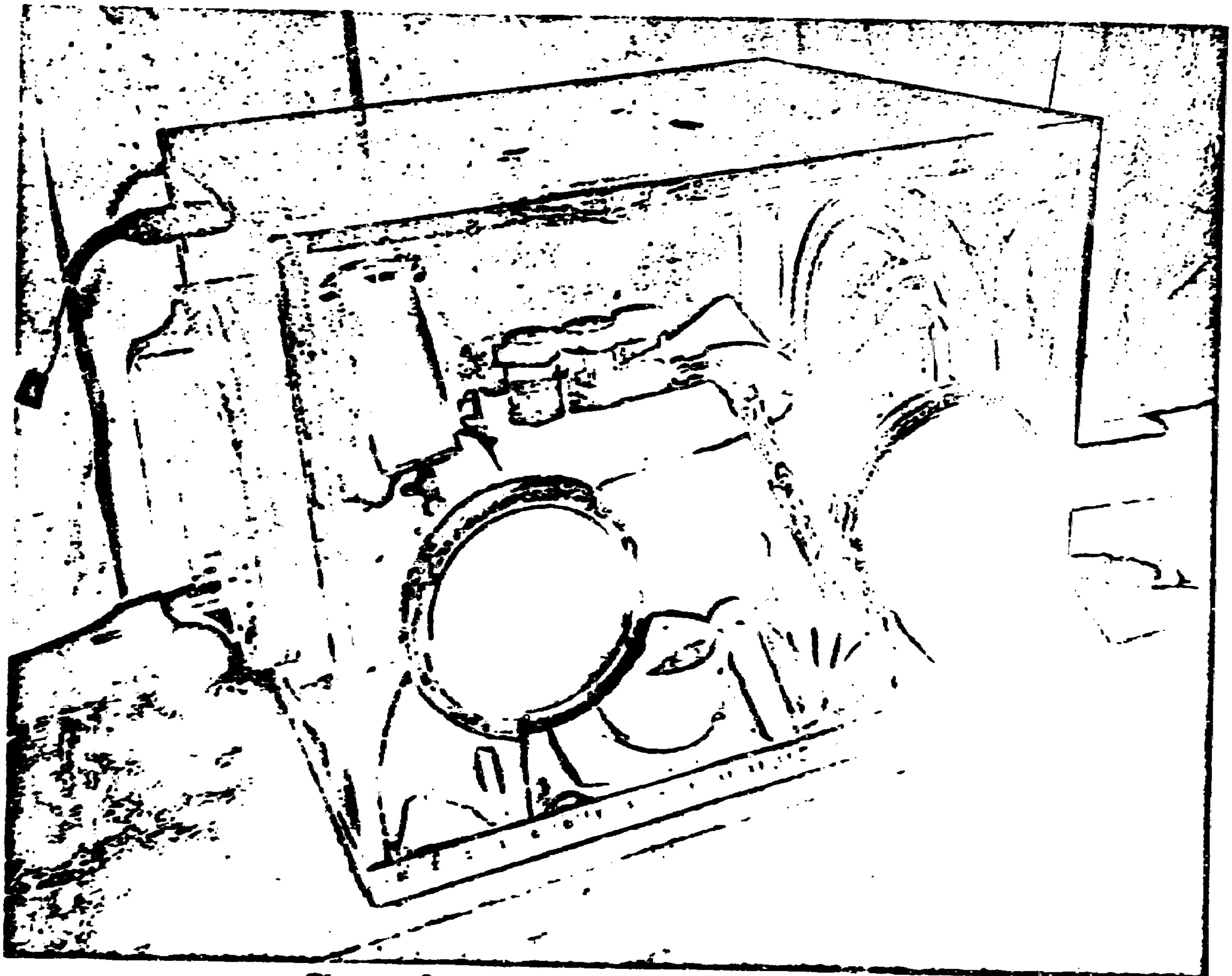

Figure 1. AIRTIGHT PLASTIC CHAMBER